

CLAIMS

1. A method of screening and recovering a regulatory DNA capable of inducing metastasis comprising the steps of:

i. transferring tagged fragments of a human DNA from malignant, metastatic cancer cells into a cell line that produces only benign, non-metastasizing tumours when injected into a syngeneic animal;

ii. injecting the transformed cells into the syngeneic animal;

iii. selecting those animals in which metastasizing tumours have been identified; and

iv. recovering the regulatory DNA capable of inducing metastasis therefrom.

2. A method as claimed in claim 1 in which the fragments of human DNA transferred in step 1 are from 0.1 to 50 kilo base pairs in length.

3. A method as claimed in claim 2 in which the fragments of human DNA transferred in step (i) are less than 1.6 kilo base pairs in length.

4. A method as claimed in claim 1, 2 or 3 in which the cell line that produces only benign non-metastasizing tumours is a rat mammary epithelial cell line.

5. A method as claimed in claim 4 wherein the rat mammary epithelial cell line is a Rama 37 cell line.

6. A method as claimed in claim 5 wherein the tag is an oligonucleotide sequence:

Primer

5' AATCCAAGCTTGC GGCCGATCAGGCCGAATATGCGGCCGCATTAT-3'
AGGTTTCGAACGCGCGCTAGTCCGGCTTATACGCGGCCGTAATATCGA

HindIII

SfiI

NorI

Defective
HindIII

AMENDED SHEET

Sub
D/15

7. ✓ A regulatory DNA capable of inducing metastasis consisting essentially of a human DNA fragment of less than 1.6 kilobase pair in length obtained from a malignant, metastasis cancer cell.

8. ✓ DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 1:

C2

CTTCCTTGGT	GCTCTATGTC	TTGCCTCTCC	CCTTCTCCAG	TCCCATTAAAG	CCATAACCAT
CTTGACAGAC	TCTGGGACAG	TCCCCTCTGC	TCTCCTGTTG	GCGCCTGAGT	CCCTTTTTCG
CTGAGGACCC	TTACCGTAGC	CTCCCATCTG	GATGACCTAG	TAGAAGACGT	GGGAAGTTGT
CACACTCAGG	TAACTGAGCA	GAGCTCAGAG	ATTTAAAGTG	AGTCTGGGGA	GCCTCGAGGA
TTGATCTGCT	GCCTTAABAA	CCCAATTGGA	TGACTAACCC	AGACTATTGT	CACTTTAGGT
GGGAAGTCAC	TAGCATATCT	GATGGGTCAC	ATCTGAGAAA	GGTTTCTAGC	AGTGGTGGCC
TTGTGTGAGC	AGCATGGCGT	GTATCATGGT	GTGCAGCATA	CTCAGGCTGC	TTGCAACACT
CGAGGCTCTT	CTTCAGTATT	AGGGGAACCA	CTGGTGTTSG	AACATGGTCC	AAGPATAACAG
TCATGTGAGG	AGAATCCCAA	TGCGTCAGGA	GAAAACGAGA	GTCTGTGACC	TCCATTCTTC
AAGATACAGA	AFTATTCTTG	GACTGTGTTT	TCATGCTCCT	TGTGGATGGG	AGTGAGTTTA
CTTCAGGTTA	ATCAGCATTC	CTTACTGTTG	GTATTCAAGT	AAATGCTTAA	ATTATCCTGG
ATATACCTCT	GTGGGAAGCA	GGTTTTTGAT	ACATGCAGCT	TGTCCTTGTG	ATTGATACTG
CTTGAACTCA	AGAGAACTTT	GCTCATGTGA	TCTTTCTTAA	CCGATGGAGT	AGAAACTGTC
TGATGCTCTC	AATAAAGTTG	GCTCTTGCAC	GAGACGTTAG	TCTGTCTCTG	TTATCTGCTC
CATTCTTCCG	CTCCACAGGC	CTCTACAGCA	CTAAACCCAC	CACCGATAGA	CTCAGTCTTT
CACTGACAAA	CATCACCAGA	GGCTCTTAAC	TGAGATTATA	AACTGTTACT	AGATGATGGG
TGGATTCGCT	CCCCAGAAAC	ATTAACATTT	ACTTGAGAGAA	CTCAAGACCC	CTTTGTAGAC
ATAACTCCCA	TGGT				

9. ✓ DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 2:

C5

ATTGCTGTGA	GCCTATTAGC	GACATTTGGT	GACGCCCCCTT	TTAAGGGGGT	AGATACAAAG
AATGGGTTGA	AATTCTGTGC	CACAAACGCT	CTCCATGTTT	TCACAAATTAC	ACTTGCAACC
TGTGGTCAGC	AGCCAGAATT	TAGGGATGTC	ATGGGACAGG	GTCCGGGAAA	GAAGGAGAG
GGTAAAGGAA	AGACAGCAGC	TTAAAGTCCA	AACAGCTCCA	GGAGACTATC	TGTAGAAATA
ACATCAGACC	ATGAGGAGAA	TTGATATCAT	TGTTTTTTCAA	TGGGTATCGC	CAAGGGGACT
TTCCATCTGA	TTAAAAATAA	TTACTGCTGG	CACTAAATCC	AATTGGAAAT	GCCCCACACA
ATTTATCTTC	CACTTCATGC	TGCTACCCATA	TGCTGACGT	GGCGGAGCAG	AAGCATTCCT
TCCCGTTCTG	ATAAATAGTA	CTTTGTAAAT	ATTTGGAGAC	GGGAGCTCTG	GTGACAGGGA
ACACGTACAA	ACCGGCCTGT	TTATCATGTT	CCCGATAGAG	GCCCTCTTTG	ACGTACAGGA
CCCCAAAACA	GTCAGGATGC	TGTGAATTTC	CTTCCATGAA	GCCTTGTTCA	CAATTAGCAA
CCATTGGAGG	AAGCAGGCTG	CACTGTCTAC	CACAAAGTGGC	ACTTTCCAAA	GAGCACACAT
ATATTGGAGC	AAGACATTTT	GCTGGCTGAC	TGGTGCTGTG	TAAGCTGATA	AACTGCTATA
TTTATTAAAC	TGGCTTTTCT	TTGAACACCC	CACTCAAGGA	AAAAAAAACA	CACTTAGGGT
GACATTATTT	GGAGATGAAG	TCTTTATAGA	GATGCTTAAG	TTTAAACGAG	ACTTTTAAAG
CCGGCTCTAT	TCCATTTTAT	GAATGGTGTC	CCTACAAAGG	AAGAAACTGG	GACAGAGGTA
TGTACACTTG	TGTGTGTGTG	AGAGACAACG	TGAGGAGCTG	AAGAGGAGCA	CGTACAAGTC
AGAGAAAGGC	TGACCCTTAT	TCACACTGAG	CAPACCAGTC	ATGTGTGGGT	CGATAGATGA
GAGTATCCCC	CAAGACTCAC	ACATTTCGAAC	GCTTGGTC		

10. ✓ DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 3:

C6

AGGACCAGAG	TTCACATCCC	ATCAAATGGC	CCAGAAGGTT	TTAATGCTGT	CTTTTGGCCC
AGGGGCGAAC	TGCACACACA	TGTGCACATA	CACTTACAGA	GACACACATT	CAGCAGCATA
AGAACACAAAT	CACAAATAAA	AAAAATCTTG	AAAAATTTTA	AGCTAAAATT	GTAAAGAAAT
AACATATATA	CAATTTTTCT	TTATTTTTTT	AAAGATTTAT	TTATTTAATG	TATATGAGTA
CACTGCCTCT	CCCTCCAGAC	ATAGCAGTAC	AGGGCATCGG	ATCCCATTAC	AGATGGTTGT
GAGCCACCAT	GTGGTTTTCAC	AGATGGTTGT	GAGCCACCAT	GTGGTTTTCAG	GAATTGAACT
CAGGACCTTT	GGAAGAGCAG	TCAGTGCTCT	TAACCTCTAA	GCCATCTCTC	CTGACCCTTA
TATACAAATTT	TAATGCTACG	TACACACAAC	TTCTCTTTCC	TTTAATGGTT	GAGATTTTTG
TCTGGAGGAG	TAAGGATPAA	GGAGGGGAAAG	AACATTGCTT	TCACATTGCA	CCAGTGGGAA
CAGCGTGTTT	AAAGTAGGAA	TGCCATGAAA	TGACTGGCCT	GCCTTCTCAT	TACTGTTCCCT
CCCCTCCTC	CTTTTAACTG	GAGCTCCTTT	ATCTAATTTA	TTAGTTTGAC	GATACCCAGG
GTTTTCTTCT	GTTTTGATCT	TTTTAAGACA	GAGACTCACC	ATATAGCCCT	GGCTGGCCTG
AAGCTCACTA	TGTAGACCAG	TCTGGCCTTG	AACTCAAAGG	AGATCTATCT	GCTTCCTAGT
GCTGGGATTA	AAGGCTTGTC	CTACCAAGTC	TGGTCTGAGG	CTTTGGAGCA	GCCTCGGTTT
TGGCCTTCTT	TAAGGATCTC	TAAGCTAGCA	GTAAGTAGCC	TAGCCATGCT	GTTGTAGGAA
GTTGTTCTGT	CATCCTGGCT	CCAGCACAAA	GGCAGTCACT	AAACGTGGGC	CTCATTTCAT
CAGAGCTGAA	TGCAAAATTCC	TTGTGCTCTT	CCTGTGTCCT	CCTGGAAC	

11. ✓ DNA consisting essentially of a regulatory DNA capable of inducing metastasis from sequence 4:

C9

AGTTGGGGAC	ACAGCTTGCT	TGATTAAAGAT	GTTTCTTGGG	AAAAGGAGTT	AAGCCTAATG
ATTTCCAATG	GAAAGGACTG	CTAATTGGGG	AGGCAATGTT	GCTTAATTGG	GACACCTGCG
GGTAATTAAA	AGCTCTCTCC	CAGTGGCCTT	TCCTGTTTTT	GGCTCTGGGA	GGCGAAGGCA
TTGAGAGGGA	TGCAGGCATT	CTAAGGGCTG	GTTCTTGCTT	TCTCCCTTCC	CCTCTGTCCA
AACTCAGTGA	GGTATCCCTG	TCTGTGCTGT	CCTTAGAGTG	CCGTCCCTGAG	GCCTTGGTGA
GTAAAGGTCT	CTGGATCTGA	GCTGCCTCAG	GGAAACGCCAT	GAGCTCATTE	GAAAGGGGAG
AACCAGGCAA	AGGTGTTGGC	TGTGACCTCA	GAATTCTGAG	GGGCAAAGGT	TCAAGGCTAA
CTCTCATTAT	AGAGCAAGTT	TGAGACTGGC	CTGGGAACAA	AAATATAAAG	TGAGTGAGGT
CATATGACAG	CACCTGAGGA	GTCCTGTCCC	TAGAGATCAT	AAGGACCTGG	CTGCTGGGGA
CTTGTTGCAG	ATGGCACTTT	GTGTGAGAG	AGGGGACCTG	CCCCAGCATG	GGAGGCCCTG
GAAGATCCTC	TGGATTAACT	GTGAACACTG	ATTGCTGCTT	TATACTCTGA	GTTGTGCTGT
TATCTGGTAC	ACATCTGCTG	GGTGAATGAG	TTCATGGGCT	TTATTTTCACT	GAGGTATTTA
CCTGAGGAGA	AAGAAGGACT	GGTGCCACAA	AGCACAGCTT	TTAAATCTGT	GGGTGTGTGAC
CCATTATGGA	CTATCATAAC	TGAGTGCAAG	TATCAAGAAT	ACTTTAGCAG	GTGGTAAAAA
GATTTTTTGA	TGCGCAACGA	CCAAAACCTGA	ACTCAAAAAT	CAAGCATGGC	ATGGATCCTG
GGTGCTCCTG	GAAGCACTTG	CCTTTACTGC	ATTGTGCGAC	TTGACGGTAG	CCTTGCTTCT
GAATGCACAA	CACGTGGGCT	TTGGGCTGCA	CAGGCCACCA	CGCCGTGCCT	GAAACACCTC
AGCTCAGGTT	TGTGGCTATG	TCCTATGACT	TGGACTTACT	TTTATTGCAC	ATATAAATAT
TTTCCTGC					

12 ✓ DNA consisting essentially of a regulatory
DNA capable of inducing metastasis from sequence 5:

C12

GAGGGGGTGG	TGGCACAGTT	ATGTTTTTGT	AGGAAGGGTT	CCATGAACCT	CAGCAGAGCT
CGGGTTAGAA	ATTTAAAAGC	CCTGAGGGGA	ATTTTTTTTTT	TAAATCGCTA	TGAATCTGAC
ATGAGAAAAA	CAGATCAGAA	ACGTTCTTGT	GCTTCAGAAA	AGGACAAGTG	TGTGAGCTAA
CAGACTGCAC	ACTGGTGCTT	GAGGCACATC	TGGATCACAG	GAGCGTCAGA	TAATGTCCCC
AAAGGTAAAT	GCATTTGCTT	GCACAGTACC	GAGTGTTGGT	GGGGGTGCCT	ACAGCCCAGC
GGTTCTCAAC	CTTCCTGATG	CTTCGACCCT	TTAATAACAGT	GCCTCATGCT	CTGGTGACCT
CCCCAACCTT	AAAATTATTT	TTGTTGCTGT	TCATAACTGT	GATTTTGATA	CTGTTATGAA
TTGTAATATA	AATAATTTTG	AAGAAAGAGG	TTTGCCAAGG	GTTTGAGAAC	TGCTGTTCTA
GCCCCACGTG	GATGGTTTTT	CGTCATTTGG	GGTTTTTTATC	AGGCAGAGTC	TTATGTAGCC
CAGGCTAGCA	GCCTAGAAATG	TGCTACTTAG	CTGAGGAATA	ACCTTGGAAC	TTCTGAGGAC
TGGAGAGACT	GGCTTAGTCC	TCAAGAAACT	GGAAATAGCT	GGAGTTTGGC	TACTTGTGGG
TTCTTTTTTC	TTCAAACCTT	TTCTACTCTT	TTTCCACCCT	GTCGGCCCCC	TAACACTAAA
TAAGAAAAG	AAAGGGGAGC	ATAGAGGGGA	AAAGAAACCC	CTGAATAACG	TCAGTAGTTG
GCAAGGGGGG	GTGACATATG	TTGTCATTAG	ACCACATCCT	GGTGATTAA	GGGAGTCAAG
TTCTTTGGGG	CAAGTTTGAT	CTTTCGTGTA	ACGATATCTA	ATTTCTTCTC	CCTGTTGCTT
CGTCTTTGTG	AACPAAGACT	TGATAACCCA	CAATGGACCA	TCAACCAACC	AACCAACCAT

13 ✓ DNA consisting essentially of a regulatory

DNA capable of inducing metastasis from sequence 6:

C20

TTGTCTCTGG	TGTTACTTGT	TTTCCCATT	CTGACAGTGG	TTTGACCTT	CTATACGCCT
GTGTGTCAGG	AGTGCTGTAG	ACCTATTTTC	CTGTTTTCTT	TCAGCCAGTT	ACAGGAACAG
AGTGTTCTAC	TGTCAGATGT	GTAGCTGTTC	CTGTCCACTG	ACTTTCAAGC	TGTCTCTGTG
TGCAGGAACC	AGAAGGGCCT	GTCCCTACTT	CTACTGGGCC	CCTACGCACA	GGGGGCCTAG
ATGGTGCTAG	GTGTTTTCTT	CTAGAGCCTG	AAATGTGGGC	AGAGAGTAGT	CTCCTCTGGT
TTCTTAGGTA	TGTCTTCCCC	TCTGAAGGTC	TAGCTCTCCC	TTCCATGGGA	TATGGGTGCA
GGGAGCTGTT	TGACCAGGTC	CTCTCAAATC	CGGGTGCACT	CTGGACCGCA	GGCTCCTGTA
GCTTGCCCTGC	TGCAATCTTC	CCGCACCCAG	AGGCACCCAA	GTTTCCTCTT	GGGCCAAGGA
TGTGGGCAAA	GGTGGGCAGA	AGTGGCAATC	TCTCCTGCCC	TAGCGTCTCA	GGATTGCCCT
CACTTCTGGG	CAATCCGCTC	TCTCTTCCAC	AGGGTTTGGG	AGCAGGGAGC	TGTGGGCCGG
TATCAGGCAA	AGGTTTGAGG	CAACCAGTTA	GAAACTGGAA	GTGTCAAGTC	CCAGAGGAAT
TTTGCCCTTTG	TGTGTCCTGA	GTCCACCCAG	CAGGTCACCT	GGAGCAGAAA	AATTGGTTTT
CCCCTCGGTC	TCAGGCCTGA	AGTTGCACCT	CAGGGTTGGC	TTTCAGCTGT	ACCTGTGGAA
AGTATGGTTT	TAAAAATCTA	AGATAGCTAT	CATGCAGCAA	GGCTTGTTGA	AAATGTCTAT
TTGGTTCCCTT	TATGACTTAC	TTTTGCTGTA	CTGAGGATCA	AACCTAGGGT	CTCAAGCAGT
CATCACAATT	CTCTGTCACT	GATCCAGCTC	CATTTCTATT	TTCTTTTGTG	CCGCGCGATC
TCTCGCCAGC	AAGAAAAACAC	GCTAGGGACA	TACGAATCCT	TGCTGCAGCC	AAAACCTTTTA
TTGAATCTTA	AGGAGAAGCC	CGCGCACCGG	ACTGGCGCGG	TTTATATACA	CCCTAGCACA
GTGCATCCAC	A				

14. The use of an osteopontin gene as a
metastasis inducing transformant.

Sub A2 15. A probe specific to a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

Sub 17 16. A kit for diagnosing the likelihood of a cancer metastasizing comprising a probe as claimed in claim 15 and one or more of a colour indicator, an oligonucleotide primer, materials for gel analysis and materials for DNA transfer or hybridisation.

Sub A3 17. A medicament adapted to target a regulatory DNA capable of inducing metastasis as claimed in any of claims 7 to 13.

add A4

insert 20